Review

# Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss

**Donald K. Layman, PhD**

*Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, Illinois*

**Key words: obesity, leucine, protein synthesis, glycemic control**

Evidence is accumulating that diets with reduced carbohydrates and increased levels of high quality protein are effective for weight loss. These diets appear to provide a metabolic advantage during restricted energy intake that targets increased loss of body fat while reducing loss of lean tissue and stabilizing regulations of blood glucose. We have proposed that the branched-chain amino acid leucine is a key to the metabolic advantage of a higher protein diet because of its unique roles in regulation of muscle protein synthesis, insulin signaling and glucose re-cycling via alanine. These metabolic actions of leucine require plasma and intracellular concentrations to increase above minimum levels maintained by current dietary guidelines and dietary practices in the U.S. Initial findings support use of dietary at levels above 1.5 g/kg · d during weight loss. Further, our research suggests that increased use of high quality protein at breakfast maybe important for the metabolic advantage of a higher protein diet.

**Key teaching points:**

- Methods for nitrogen balance and amino acid oxidation are well suited for defining minimum requirements for essential amino acids but of limited use in understanding optimum amino acid needs for adult health.
- Leucine is an essential amino acid with multiple metabolic roles beyond the fundamental use as a substrate for synthesis of new proteins.
- Leucine may be a key regulatory amino acid for maintenance of muscle mass during catabolic periods such as weight loss.

## INTRODUCTION

The optimum protein intake for weight loss diets remains unknown. Many of the current diet approaches focus on reducing carbohydrates with dietary fat and protein added primarily as energy "fillers" [1–3]. However, there is increasing evidence that protein intakes above the current RDA may be beneficial during weight loss. Early evidence in support of higher protein intakes was derived from studies using very low calorie diets [4]. These investigators found that increasing dietary protein to levels of 1.5 g protein per kilogram of ideal body weight reduced loss of lean tissue during rapid weight loss. Other researchers have suggested that there is a metabolic advantage with a high protein, low carbohydrate diet that may be associated with increase thermogenesis [5]; or that protein has a higher satiety value reducing net food intake [6,7]. We proposed that increased dietary protein contributes to a mix of metabolic outcomes beneficial to weight loss and that the branched-chain amino acid leucine may be a critical predictor of protein quantity and quality for food choices during weight loss [8–10]. Our approach suggests there is an important difference between amino acid roles in meeting a minimum protein requirement versus "optimum metabolic needs".

## MINIMUM REQUIREMENTS VERSUS OPTIMUM INTAKES

Traditional approaches to defining human nutrient requirements evolved from concepts based on preventing deficiencies and maintaining efficient growth. The first nutrition guidelines focused on minimum daily requirements necessary to prevent frank deficiencies [11]. For vitamins, intakes were designed to prevent deficiency conditions such as beriberi or scurvy; while

Address reprint requests to: Donald K. Layman, Ph.D., 437 Bevier Hall, University of Illinois, 905 South Goodwin, Urbana, IL 61801, E-mail: dlayman@uiuc.edu
Support for this research provided by the Illinois Council on Food and Agriculture Research, Kraft Foods and Cattlemen's Beef Board and National Cattlemen's Beef Association.

Journal of the American College of Nutrition, Vol. 23, No. 6, 631S–636S (2004)
Published by the American College of Nutrition

for protein the deficiency conditions were kwashiorkor and marasmus. Early on, researchers recognized that very little protein was required to prevent these deficiencies and that these levels were far below the levels of protein consumed in most industrialized countries. While the minimum amount of protein needed to prevent frank deficiency was low, it was also recognized that the amino acid composition of the protein (protein quality) and the energy content of the diet influenced the quantity of protein needed and the efficiency of utilization [12].

In the early 1940's, nutrition thinking moved from minimum requirements to the more general concept of Recommended Dietary Allowances (RDA). The RDA for protein was defined as *the level of protein judged to be adequate . . . to meet the known nutrient needs for practically all healthy people* [13]. Based on the information available, the Food and Nutrition Board set the RDA at two standard deviations above the average requirement to meet the minimum needs of 97.5% of the population.

The primary measurements used to define average protein requirements are short-term nitrogen balance and amino acid oxidation [14,15]. The primary focus is protein utilization and how to achieve maximum growth or maintenance of lean body mass using the least dietary protein. The experimental methods were based on feeding a range of protein intakes and monitoring changes in nitrogen balance or amino acid oxidation. These measures exhibit an inflection point believed to reflect the minimum protein intake necessary to maintain maximum lean body mass.

While an inflection point in the nitrogen balance graph is usually clear, the nitrogen balance values above the inflection point are not usually zero as the concept implies. In 1978, Hegsted [16] reviewed the literature on human nitrogen balance studies including studies ranging up to one year in length. He found that the nitrogen balance graph remained positive at nitrogen intakes above the inflection point. Most investigators point to these data as evidence for methodology flaws in the nitrogen balance approach. So while researchers have dogmatically used the nitrogen balance approach to predict the minimum protein requirement, they have been unwilling to accept the complete data set that protein intakes above the inflection point result in net nitrogen retention and maintenance of higher levels of lean body mass.

Similar findings have been obtained from monitoring changes in plasma amino acid concentrations and the rate of amino acid oxidation [14,17]. These experiments were designed similar to nitrogen balance studies with increasing levels of dietary protein while investigators monitor changes in plasma amino acids. At low protein intakes, plasma amino acid concentrations remain relatively stable and amino acid oxidation is low. As protein intake increases there is an inflection point similar to the nitrogen balance data. At the inflection point, the concentration of plasma amino acids increases followed closely by increases in rates of amino acid oxidation. Similar to nitrogen balance, this inflection point has been defined as the minimum protein intake necessary to maintain maximum efficiency of amino acid use. Underpinning this concept is the assumption that amino acid oxidation provides no metabolic or physiological advantage. From an animal sciences perspective, this inflection point represents that most cost effective diet for growth. So production agriculture limits the use of expensive protein and uses less expensive carbohydrates to maximize energy intake and total weight gain. While these concepts are fundamental to the cost/benefit ratio for production agriculture, it is unclear that the same logic holds for defining the optimum protein intake for adult health in the U.S.

In 1994, the Food and Nutrition Board began to emphasize that for any nutrient there is a range of dietary intakes to support optimal metabolic needs. This concept of an optimal range is reflected in the Dietary Reference Intakes [18,19]. The DRI recognize that metabolic needs range from a minimum level necessary to prevent deficiencies (the current RDA) to an upper limit (UL) where higher intakes may produce adverse effects of excess or toxicity. For vitamins and minerals, the DRI concept of range is readily accepted; however, for the macronutrients the concept of optimal metabolic range or UL remains virtually unexplored.

Application of the DRI concept of range of intake to the macronutrients is complicated by the diversity of their functions. The protein requirement of 0.8 g/kg.d reflects a composite need for twenty amino acids. While eleven of the amino acids are considered dispensable (non-essential) because they can be made in the body from nitrogen provided by other amino acids, the remaining nine amino acids are indispensable (essential) and must be provided daily in the proper amounts. The needs for each of these indispensable amino acids vary with their roles in metabolism. Two of the indispensable amino acids, lysine and leucine, illustrate the range of metabolic differences among these amino acids.

Nitrogen balance is a concept particularly useful for defining a minimum RDA for a limiting amino acid such as lysine that serves as an essential amino acid for peptide structures but has limited use as a metabolic substrate [20,21]. On the other hand, leucine, one of the branched-chain amino acids (BCAA), is required for numerous metabolic processes. Leucine's roles in metabolism range from the fundamental role as a substrate for protein synthesis to a modulator of insulin signaling [22–24] and a critical nitrogen donor for synthesis of alanine and glutamine [25,26]. The potential for leucine to participate in each of these metabolic processes appears to be in proportion to availability. Experimental evidence comparing the priority for use of leucine in each of these individual processes is limited, but suggests that the first priority is for aminoacylation of tRNA for protein synthesis [27], while the influence of leucine on the insulin signaling pathway is dependent on increasing intracellular concentrations [28].

## METABOLIC ROLES OF LEUCINE

Regulatory roles for leucine in muscle metabolism were first reported for protein synthesis. During catabolic periods such as fasting or energy restriction, supplementation with leucine or a complete mixture of the three BCAA, leucine, isoleucine, and valine, stimulates muscle protein synthesis [37–39]. Likewise, leucine supplementation stimulates recovery of muscle protein synthesis after exercise [40,41]. The molecular mechanisms for the actions of leucine in protein synthesis are now known to involve regulation of phosphorylation events and components of the insulin signaling pathway. The site for leucine action is a kinase in the insulin signaling cascade previously identified as mTOR (mammalian target of rapamycin). This regulation was first recognized associated with translational control of muscle protein synthesis [23,40]. Increases in leucine concentration stimulate mTOR kinase activity for phosphorylation control of the eIF4 initiation complex and of the S6 ribosomal protein. Specifically, leucine stimulates phosphorylation of the inhibitory binding protein 4E-BP1 causing the binding protein to dissociate from the eIF4E translational initiation factor. After dissociation, eIF4E is available to bind with eIF4G and form the active initiation complex. Leucine via mTOR also increases activation of $p70^{S6}$ kinase leading to phosphorylation of the S6 ribosomal protein and enhanced global rates of protein synthesis [42]. The mechanisms for translational regulations by leucine have been recently reviewed [24,42]. This unique role of leucine in regulation of muscle protein synthesis is consistent with the sparing of lean body mass seen with use of higher protein diets during weight loss [2,10].

The oxidative pathway for leucine is also dependent on intracellular concentrations and illustrates a second unique metabolic role of the BCAAs. Catabolism of most amino acids occurs in the liver facilitating disposal of the amino-nitrogen in the urea cycle. However, metabolism of leucine and the other two branched-chain amino acids (BCAA) valine and isoleucine occurs predominately in skeletal muscles [31] because the liver lacks the aminotransferase enzyme required to initiate BCAA metabolism. This is a striking metabolic difference for these amino acids which becomes even more remarkable with the realization that the three BCAA account for over 20% of total dietary protein. Using the traditional thinking that protein requirements should be defined by measurements of optimal efficiency of nitrogen handling we are left to ponder why the body evolved to metabolize 20% of total amino acids (and total nitrogen) in a peripheral tissue? A likely explanation is that the BCAA are providing the amino-groups for production of glutamate, alanine and glutamine in skeletal muscle [8,31]. These amino acids are important for balancing substrates for the TCA cycle within skeletal muscle [43] and as substrates for gluconeogenesis in liver [44].

Daily requirements for leucine are currently established at 1 to 3 g/d [29,30]. These requirements are based on nitrogen balance and amino acid oxidation methods that target minimum levels of amino acids for protein synthesis. When the minimum need for protein synthesis is met; intracellular concentrations rise in proportion to dietary intake; and leucine is available to impact the signaling pathway and muscle protein synthesis and to contribute to production of alanine and glutamine. These roles are dependent on increasing plasma and intracellular concentrations [31–33]. Based on studies evaluating recovery after exercise or short-term fasting, stimulation of muscle protein synthesis appears to require a minimum of 18 g of a complete mixture of the essential amino acids or a minimum 2.5 g of leucine to increase intracellular concentrations [45–47]. To optimize these metabolic pathways, we estimate leucine use at 7 to 12 g/d [34–36].

To maximize the impact of protein on metabolic regulations, critical factors for diet decisions are likely to include amino acid content (protein quantity) and amino acid ratios (protein quality), as well a the distribution of protein throughout the day. The need for distribution of dietary protein throughout the day is another concept this is virtually untested. We know that diurnal rates of muscle protein synthesis are lowest after an overnight fast [48]. Further, the anabolic impact of an individual meal is likely to be 5–6 hr based on the rate of amino acid metabolism after a meal [49]. Hence we hypothesized that the most critical meal would be the breakfast meal after a 12-hr overnight fast and that dietary protein should be provided at approximately 5-hr intervals throughout the day. So, while many Americans consume a large portion of their daily protein at a meal late in the day, we targeted a minimum of 30 g of protein for breakfast with more balanced distribution throughout the day.

## DIETARY PROTEIN IN WEIGHT LOSS STUDIES

Using these concepts of protein quantity, quality and distribution, we designed a weight loss diet with 1700 kcal/d that provided 10 g/d of leucine with a minimum of 2.5 g of leucine at each meal (designated a PRO group). For a control comparison, we used the diet recommendations as defined by the USDA Food Guide Pyramid [50] that provided approximately 5 g/d of leucine at 1700 kcal (designated a CHO group). Assuming a leucine content of protein at 8% (range: 7% to 10%), these leucine targets require daily intake of 125 g/d of total protein in the PRO group (1.6 g/kg.d) and about 65 g/d protein in the CHO group (0.8 g/kg.d). These diets were developed using a gram-for-gram substitution of high quality dietary proteins found in eggs, dairy and meats for high carbohydrate foods such as breads, potatoes, rice and pasta. Both diets were equal in energy (1700 kcal/d), total fat (~55 g/d) and dietary fiber (~21 g/d) [9,10]. Breakfast meals for the two diets are presented in Table 1. The CHO meal was designed to represent a common American breakfast using cereal, bread

**Table 1.** Breakfast Meal

| | | | | |
|---|---|---|---|---|
| CHO Group breakfast: | | | | |
| oat cereal | | | (1 cup) | |
| milk, 3.3% | | | (4 oz) | |
| bagel | | | (1 oz) | |
| cream cheese | | | (1 Tbsp) | |
| orange juice | | | (6 oz) | |
| energy | CHO | protein | leucine | fat |
| 1.61 MJ | 57 g | 12 g | 0.76 mg | 12 g |
| | 59% | 13% | | 28% |
| Protein Group breakfast: | | | | |
| egg | | | (1 large) | |
| canadian bacon | | | (2 oz) | |
| cheese, low fat | | | (1 slice) | |
| toast | | | (1 oz) | |
| milk, 1% | | | (8 oz) | |
| energy | CHO | protein | leucine | fat |
| 1.69 MJ | 39 g | 33 g | 2.70 mg | 13 g |
| | 38% | 33% | | 29% |

and fruit juice. This breakfast provided approximately 55–60 g carbohydrates and 12–15 g protein. These values are consistent with the high carbohydrate, low fat, and low protein guidelines of the USDA Food Guide Pyramid. For the PRO group, diets emphasized intake of high quality, low fat protein foods including eggs, low fat dairy and lean meats. The PRO group breakfast provided approximately 35–40 g carbohydrate and 30–33 g protein.

After using these diets for 10 wks, women in the PRO group tended to lose more weight, more body fat and less lean mass than women in the CHO group. In total, the higher protein diet was more effective at improving body composition than the traditional recommendation for a high carbohydrate diet [10]. These findings are similar to other reports [1,2,51,52] and support the hypothesis that increased dietary leucine can spare lean tissue during weight loss.

Metabolic differences between the diet groups were seen in changes in fasting glucose and in post-prandial changes in amino acids and insulin [9,10]. After the 12-hr overnight fast, subjects in both diet treatment groups had similar plasma levels of indispensable amino acids, however subjects in the CHO group had higher levels of alanine and lower plasma glucose concentrations. These findings are similar to results observed in animal studies that demonstrate high CHO diets inhibit hepatic gluconeogenesis and reduce fasting blood glucose [53,54]. Two hours after the breakfast meal, the PRO group had increases in plasma amino acid concentrations while plasma amino acids in the CHO group were similar to 12-hr fasted values. As stated earlier, the RDA level of protein is established to minimize post-prandial increases in plasma amino acids that increase amino acid oxidation. On the other hand, the higher protein diet designed to stimulate leucine metabolism increased plasma leucine concentration approximately 2-fold after the breakfast meal [9]. This increase in leucine concentration is consistent with increases in muscle protein synthesis seen in animals during recovery after food restriction [39,41,42] and in humans after exercise [45,46]. Likewise, the increase in leucine would be expected to increase leucine oxidation and production of alanine and glutamine as substrates for gluconeogenesis [43,44].

The impact of supplemental amino acids and carbohydrates on muscle protein synthesis was further evaluated by Volpi et al. [47,55,56]. They reported age-related differences in how subjects responded to protein and carbohydrates [55]. In both young adults (~30 y.o.) and older adults (~69 y.o.) supplemental essential amino acids produced an anabolic effect on muscle protein synthesis [56]. Further, combination of the essential amino acids with carbohydrates produced an additive enhancement of muscle protein synthesis in the young adults. However, in older adults, addition of carbohydrates (producing increased plasma insulin) eliminated the anabolic effect of supplemental amino acids. These authors suggested that the presents of carbohydrates in nutritional supplements may impair the anabolic effect of muscle protein synthesis in older adults [47].

In summary, increasing dietary levels of high quality proteins while reducing carbohydrates appears to be effective for improving changes in body composition during weight loss. The increase in dietary protein resulting in increased plasma levels of leucine is consistent with molecular mechanisms for increased protein synthesis in skeletal muscle and stimulation of the glucose-alanine cycle [8,52]. These changes appear to contribute to a metabolic advantage during weight loss. These findings are consistent with other reports of a metabolic advantage for weight loss associated with diet containing reduced levels of carbohydrates and increased levels of high quality protein [5]. Additional research is needed to determine if the BCAA leucine is the critical factor in defining metabolic roles of dietary amino acids at levels above the minimum intakes defined by the RDA's.

## REFERENCES

1. Westman EC, Yancy WS, Edman JS, Tomlin KF, Perkins CE: Effect of 6-month adherence to a very low carbohydrate diet program. Am J Med 113:30–36, 2002.
2. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM: Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. Am J Clin Nutr 78:31–39, 2003.
3. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed S, Szapary PO, Rader DJ, Edman JS, Klein S: A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 348: 2082–2090, 2003.
4. Bistran BR, Winterer J, Blackburn GL, Young V, Sherman M:

Effect of a protein-sparing diet and brief fast on nitrogen metabolism in mildly obese subjects. J Lab Clin Med 89:1030–1035, 1997.
5. Feinman RD, Fine EJ: Thermodynamics and metabolic advantage of weight loss diets. Metab Syn Relat Dis 1:209–219, 2003.
6. Hill JH, Blundell JE: macronutrients and satiety: The effects of a high-protein or high-carbohydrate meal on subjective motivation to eat and food preferences. Nutr Behav 3:133–144, 1986.
7. Anderson GH, Moore SE: Dietary proteins in the regulation of food intake and body weight in humans. J Nutr 134:974S–979S, 2004.
8. Layman DK: The role of leucine in weight loss diets and glucose homeostasis. J Nutr 133:261S–267S, 2003.
9. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J: Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. J Nutr 133:405–410, 2003.
10. Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, Christou DD: A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J Nutr 133:411–417, 2003.
11. Filer LJ: Recommended dietary allowances: how did we get where we are? Nutrition Today September, p. 25, 1991.
12. Munro HN: Protein hydrolysates and amino acids. In White P (ed): “Total Parenteral Nutrition.” Acton, MA: Publishing Sciences Group. Inc., pp 59–79, 1974.
13. National Research Council: “Recommended Dietary Allowances. Protein and Amino Acids,” 10th ed. Washington DC: National Academy Press, pp. 52–77, 1989.
14. Munro HN, Crim MC: The protein and amino acids. In Shils ME, Young VR (eds): “Modern Nutrition in Health and Disease,” 7th ed. Philadelphia: Lea & Febiger, pp 1–37, 1988.
15. Millward DJ: Macronutrient intakes as determinants of dietary protein and amino acid adequacy. J Nutr 134:1588S–1596S, 2004.
16. Hegsted DM: Assessment of nitrogen requirements. Am J Clin Nutr 31:1669–1677, 1978.
17. Pencharz PB, Ball RO: Different approaches to define individual amino acid requirements. Annu Rev Nutr 23:101–116, 2003.
18. Food and Nutrition Board: “How Should the Recommended Dietary Allowances Be Revised?” Washington DC: National Academy Press, 1994.
19. Institute of Medicine, Food and Nutrition Board: “Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids.” Washington DC: National Academy Press, 2002.
20. Waterlow JC, Garlick PJ, Millward DJ: “Protein Turnover in Mammalian Tissues and in the Whole Body.” Amsterdam: Elsevier-North Holland, 1978.
21. Wolfe RR, Wolfe MH, Nadel ER, Shaw JHF: Isotopic determination of amino acid-urea interactions in exercise in humans. J Appl Physiol 56:221–229, 1984.
22. Patti M-E, Brambilla E, Luzi L, Landaker EJ, Kahn CR: Bidirectional modulation of insulin action by amino acids. J Clin Invest 101:1519–1529, 1998.
23. Xu G, Kwon G, Marshall CA, Lin T-A, Lawrence JC: Branched-chain amino acids are essential in the regulation of PHAS-I and p70 S6 kinase by pancreatic $\beta$-cells. J Biol Chem 273:28178–28184, 1998.
24. Kimball SR, Jefferson LS: Regulation of protein synthesis by branched-chain amino acids. Curr Opin Clin Nutr Metab Care 4:39–43, 2001.
25. Ahlborg G, Felig P, Hagenfeldt R, Wahren J: Substrate turnover during prolonged exercise in man. J Clin Invest 53:1080–1090, 1974.
26. Ruderman NB: Muscle amino acid metabolism and gluconeogenesis. Ann Rev Med 26:245–258, 1975.
27. Tischler ME, Desautels M, Goldberg AL: Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis in degradation in skeletal and cardiac muscle? J Biol Chem 257: 1613–1621, 1982.
28. Lynch CH, Hutson SM, Patson BJ, Vaval A, Vary TC: Tissue-specific effects of chronic dietary leucine and norleucine supplementation on protein synthesis in rats. Am J Physiol 283:E824–E835, 2002.
29. FAO/WHO/UNU: Energy and protein requirements. Report of joint FAO/WHO/UNU expert consultation. WHO Tech Pep Ser 724:1–206, 1985.
30. Millward D, Fereday A, Gibson N, Pacy P: Aging, protein requirements, and protein turnover. Am J Clin Nutr 66:774–786, 1997.
31. Harper AE, Miller RH, Block KP: Branched-chain amino acid metabolism. Annu Rev Nutr 4:409–454, 1984.
32. Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J: Substrate turnover during prolonged exercise in man. J Clin Invest 53:1080–1090, 1974.
33. Anthony JC, Yoshizawa F, Gautsch-Anthony T, Vary TC, Jefferson LS, Kimball SR: Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 130:2413–2419, 2000.
34. El-Khoury AE, Kukagawa NK, Sanchez M, Tsay RH, Gleason RE, Chapman TE, Young VR: The 24-h pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults. Am J Clin Nutr 59:1012–1020, 1994.
35. Evans WJ, Fisher EC, Hoerr RA, Young VR: Protein metabolism and endurance exercise. Physician Sports Med 11:63–72, 1983.
36. Pencharz PB, Ball RO: Amino acid needs for early growth and development. J Nutr 134:1566S–1568S, 2004.
37. Li JB, Jefferson LS: Influence of amino acid availability on protein turnover in perfused skeletal muscle. Biochim Biophys Acta 544: 351–359, 1978.
38. Buse MG, Reid SS: Leucine. A possible regulator of protein turnover in muscle. J Clin Invest 56:1250–1261, 1975.
39. Hong SC, Layman DK: Effects of leucine on in vitro protein synthesis and degradation in rat skeletal muscle. J Nutr 114:1204–1212, 1984.
40. Gautsch TA, Anthony JC, Kimball SR, Paul GL, Layman DK, Jefferson LS: Availability of eIF-4E regulates skeletal muscle protein synthesis during recovery from exercise. Am J Physiol 274:C406–C414, 1998.
41. Anthony JC, Yoshizawa F, Gautsch-Anthony T, Vary TC, Jefferson LS, Kimball SR: Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 130:2413–2419, 2000.
42. Anthony JC, Anthony TG, Kimball SR, Jefferson LS: Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. J Nutr 131:856S–860S, 2001.

43. Wagenmaker AJM: Protein and amino acid metabolism in human muscle. Adv Exp Med Biol 441:307–319, 1998.
44. Layman DK, Baum JI: Dietary protein impact on glycemic control during weight loss. J Nutr 134:968S–937S, 2004.
45. Biolo G, Tiption KD, Klein S, Wolfe RR: An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol 273:E122–E129, 1997.
46. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, Wolfe RR: An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 88:386–392, 2000.
47. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR: Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 78:250–258, 2003.
48. Garlick PJ, Clugston GA, Swick RW, Waterlow JC: Diurnal pattern of protein and energy metabolism in man. Am J Clin Nutr 33:1983–1986, 1980.
49. Hellerstein MK, Neese RA, Linfoot P, Christiansen M, Turner S, Letscher A: Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. J Clin Invest 100:1305–1319, 1997.
50. US Department of Agriculture/Department of Health and Human Services: "Dietary Guidelines for Americans," 4th ed, Home and Garden Bulletin 232. Washington DC: DHHS, 1995.
51. Piatti PM, Monti LD, Magni F, Fermo I, Baruffaldi L, Nasser R, Santambrogio G, Librenti MC, Galli-Kienle M, Pontiroli AE, Pozza G: Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high carbohydrate diet. Metab 43:1481–1487, 1994.
52. Parker B, Noakes M, Luscombe N, Clifton P: Effect of a high protein, high monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. Diabetes Care 25:425–430, 2002.
53. Rossetti L, Rothman DL, DeFronzo RA, Shulman GI: Effect of dietary protein on in vivo insulin action and liver glycogen repletion. Am J Physiol 20:E212–E219, 1989.
54. Kabir M, Rizkalla SW, Qhignard-Boulange A, Guerre-Millo M, Boillot J, Ardouin B, Luo J, Slama G: A high glycemic index starch diet affects lipid storage-related enzymes in normal and to a lesser extent in diabetic rats. J Nutr 128:1878–1883, 1998.
55. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR: The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. J Clin Endocrinol Metab 85:4481–4490, 2000.
56. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR: Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. Am J Physiol 277:E513–E520, 1999.

*Received October 8, 2004.*